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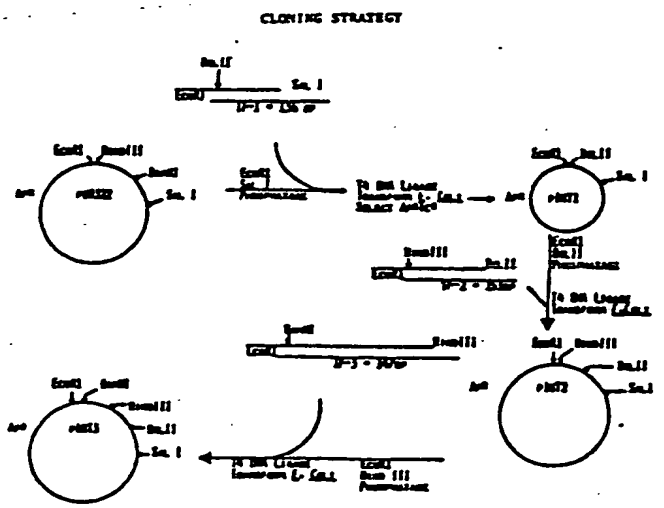
(54) Title: THE MANUFACTURE AND EXPRESSION OF LARGE STRUCTURAL GENES

(57) Abstract



Rapid and highly efficient procedures for the total synthesis of linear, double stranded DNA sequences in excess of about 200 base pairs in length, which sequences may comprise entire structural genes. Novel sequences are prepared from two or more DNA subunits provided with terminal regions comprising restriction endonuclease cleavage sites facilitating insertion of subunits into a selected vector for purposes of amplification during the course of the total assembly process. The total, finally-assembled sequences include at least one, and preferably two or more, unique restriction endonuclease cleavage site(s) at intermediate positions along the sequence, allowing for easy excision and replacement of subunits and the corresponding b· facile preparation of multiple structural analogs of polypeptides coded for by the sequences. Manufactured genes preferably include codons selected from among alternative codons specifying the same amino acid on the basis of preferential expression in a projected host microorganism (e.g., *E. coli*) to be transformed. Illustrated is the preparation and expression of manufactured genes capable of directing synthesis of human immune and leukocyte interferons and of other biologically active proteinaceous products, which products differ from naturally-occurring forms in terms of the identity and/or relative position of one or more amino acids, and in terms of one or more biological and pharmacological properties but which substantially retain other such properties.